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An Automated Device (AGARS) for Studying Avoidance of Pollutant Gradients by Aquatic Organisms

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Most apparatus designed to detect avoidance of pollutants by aquatic organisms require visual observations of test organisms in steep pollutant gradients. GAARS (Aquatic Gradient Avoidance Response System) was developed to eliminate these limitations. This system allows animals to choose between one uncontaminated zone and three increasingly toxic zones in a gradient trough that is monitored for extended time periods by infrared light sources, sensors, and a microprocessor. Data are accumulated hourly and processed by a paper tape reader/calculator/plotter system that records the time test animals remain in each zone and compares behavior before and during test exposures. Initial tests indicate that pinfish (*Lagodon rhomboides*) tested in the apparatus will avoid chlorine-produced oxidants at concentrations of 0.02-0.04 mg/L.

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An Automated Device (AGARS) for Studying Avoidance of Pollutant Gradients by Aquatic Organisms[1]

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Most apparatus designed to detect avoidance of pollutants by aquatic organisms require visual observations of test organisms in steep pollutant gradients. AGARS (Aquatic Gradient Avoidance Response System) was developed to eliminate these limitations. This system allows animals to choose between one uncontaminated zone and three increasingly toxic zones in a gradient trough that is monitored for extended time periods by infrared light sources, sensors, and a microprocessor. Data are accumulated hourly and processed by a paper tape reader/calculator/plotter system that records the time test animals remain in each zone and compares behavior before and during test exposures. Initial tests indicate that pinfish (*Lagodon rhomboides*) tested in the apparatus will avoid chlorine-produced oxidants at concentrations of 0.02–0.04 mg/L.

Key words: avoidance responses, behavioral bioassays, automated monitoring systems, chlorine-produced oxidants

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La plupart des appareils servant à détecter l'évitement des contaminants par les organismes aquatiques requièrent l'observation visuelle des organismes expérimentaux dans des gradients prononcés de contaminants. Nous avons mis au point un système, l'AGARS (« Aquatic Gradient Avoidance Response System »), qui n'est pas soumis à ces contraintes. Grâce à ce système, les animaux peuvent choisir entre une zone non contaminée et trois zones de toxicité croissante dans un bassin à gradient pouvant être surveillé durant de longues périodes à l'aide de sources de lumière infrarouge, de détecteurs et d'un organe de micro-traitement. Les données sont accumulées d'heure en heure et traitées au moyen d'un système comprenant un lecteur de bande perforée, une calculatrice et un traceur, qui enregistre le temps passé par les animaux expérimentaux dans chaque zone et qui compare leur comportement avant et durant les expériences. Des essais préliminaires indiquent que des *Lagodon rhomboides* placés dans l'appareil évitent des oxydants produits par le chlore à des concentrations de 0,02 à 0,04 mg/L.

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THE avoidance of pollutants by fish and invertebrates can cause their absence from the pollutant source (Sprague et al. 1965; Fava and Tsai 1976; Laughlin et al. 1978). Lack of avoidance could result in the death of an organism or bioconcentration of harmful substances. Laboratory tests that determine an organism's threshold of avoidance or preference for a pollutant can thus be an aid in determining the impact of the pollutant. The adaptive significance of fish locomotor orientation behavior and approaches to quantifying and forecasting responses to environmental stimuli (i.e. temperature, light, or chemicals) have been addressed by Kleerekoper (1977).

Most avoidance tests involve the visual observation of animals during daylight hours, for less than 30 min, in Y-shaped troughs (Laughlin et al. 1978), rectangular troughs (Lewis and Livingston 1977), or cylinders (Middaugh et al. 1977a). Short-term visual monitoring may not provide information about long-term exposure. Long-term observations might reveal that organisms acclimate to a toxicant or react differently to a pollutant in darkness. For example, a pollutant's turbidity (Lawrence and Scherer 1974) or difference in color from the surrounding water may not be detected by organisms in darkness.

Most avoidance tests present a very steep gradient, rather than the shallow gradient likely to occur in the field. Several investigators have indicated that the response to steep and shallow toxicant gradients are not always the same (Timms and Kleerekoper 1972; West-

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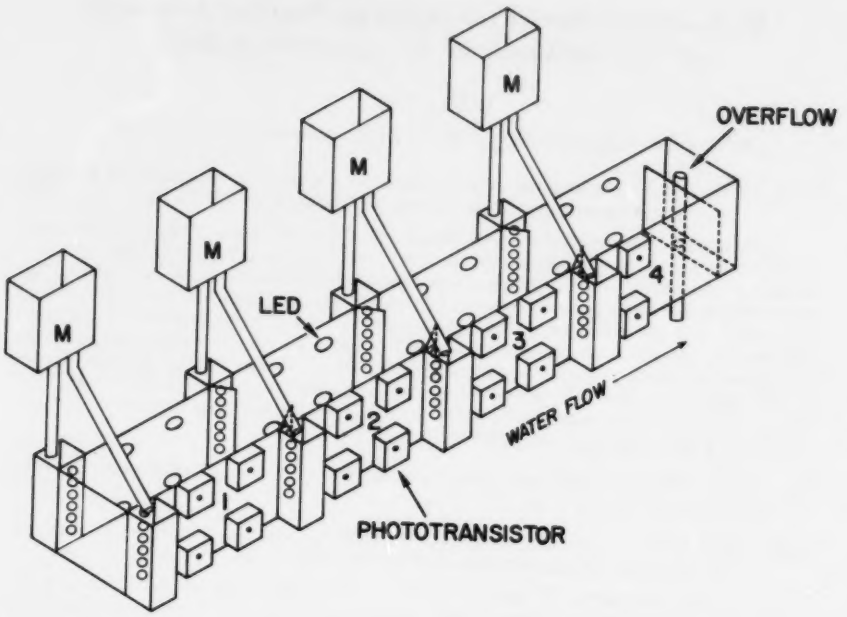

FIG. 1. Diagram of AGARS (Aquatic Gradient Avoidance Response System) trough; for a full description of dimensions see text. Organism position can be monitored in both the upper and lower half of each area (1–4) by pairs of infrared light-emitting diodes (LEDs) and phototransistors.

lake and Kleerekoper 1974). Results have usually been obtained by testing water with one pollutant concentration vs. water free of the pollutant, requiring a series of tests to establish a threshold avoidance concentration.

A system described by Kleerekoper et al. (1970) solves some of these problems. It monitors the orientation of test organisms to pollutant gradients in a 5-m square tank with 1936 photoconductive cells and an on-line computer. This method, however, is expensive. In addition, its light sources (at a color temperature of 2400°) would radiate a significant amount of visible light (Koller 1952) which may affect nocturnal activity patterns. Thus, the need exists for a relatively inexpensive system that produces a gradient, functions automatically, and is easily operated.

The system described here, an Aquatic Gradient Avoidance Response System (AGARS), produces a pollutant gradient in a continuous-flow system, providing the test animal with a choice between uncontaminated water and three pollutant concentrations in a rectangular trough. The position of the organism in the trough is monitored by infrared lights (peak emission 940 nm, range 860–1060 nm) to avoid affecting the organism's photoperiod response; sensors are scanned at preset intervals by an inexpensive microprocessor. Cumulative data, periodically punched on paper tape by a teletypewriter, can be analyzed manually or by calculators or computers equipped with a tape reader. Thus, a relatively large amount of data on avoidance behavior can be manipulated, avoiding the cost of an expensive on-line computer. Results of a test with a chlorine-produced oxidant gradient illustrates the advantages of a system that eliminates the need for visual observations and steep gradients.

Materials and Methods

The gradient trough (Fig. 1) is constructed of clear 6-mm plexiglass with inner dimensions of 125 × 17 × 15 cm high. A peristaltic pump injects a test compound and carriers, when required, into a continuous supply of 1.5 L of seawater/min before flowing into plexiglass mixing boxes. Mixing is accomplished through turbulence. This flow is divided evenly between pairs of small chambers along opposite sides of the gradient trough. Water enters the main trough area through a vertical row of six 7-mm holes designed to control water flow. Vertical strips of plexiglass (at a 45° angle to the side of the trough) channel incoming water toward the drain. Water flow maintains a gradient consisting of a control zone and zones that receive three increasing toxicant concentrations. By adding increasing

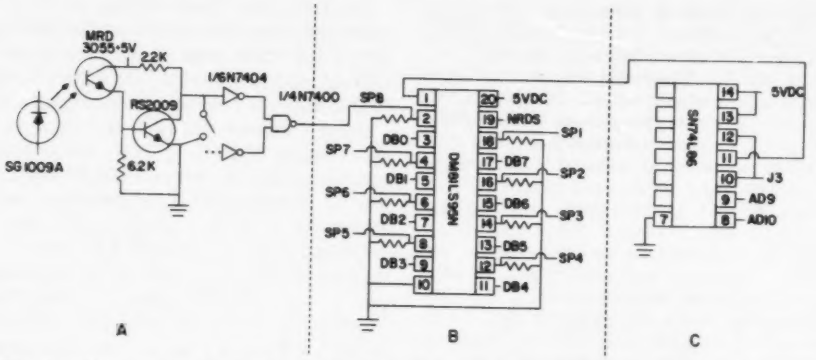

FIG. 2. Diagram of circuits for monitoring positions of organisms within gradient trough: A, Phototransistor circuit; B, Octal buffer circuit; C, IC circuit for addressing octal buffer. SP1–8 represent the digitalized outputs from the eight sensor pairs in Part A. DBØ-7, NRDS, and AD 9–10 are connected to the corresponding pins of the microprocessor chip. J3 is marked on the microprocessor printed circuit board. The eight resistors in B are 10K, ¼ W.

toxicant concentrations to Areas 1–4, the gradient increases as it flows downstream. To reverse the gradient, toxicant addition to Area 1 produces a gradient that decreases downstream due to dilution with clean water from Areas 2 to 4. The same organisms can be subjected to both gradients on different days to eliminate possible bias due to end effects or rheotaxis.

An opaque, plastic curtain, enclosing the gradient trough, eliminates visual disruptions. Photoperiods are maintained by a timer (12L:12D) controlling two 40-W fluorescent lights. Filtered seawater is temperature-controlled and its salinity is monitored. Animals are fed frozen adult brine shrimp (free from significant PCB or common organochlorine pesticide residues) at irregular intervals through tubes that penetrate the plastic curtain, thus preventing anticipation of food. Food enters the trough at three points between each of the four areas. Animals are normally not fed during the 24-h period when a toxicant gradient is present. Water is sampled from the centers of the four areas for analysis using a glass siphon with a Teflon® stopcock. Each area's siphon normally rests near the surface and is gently lowered from outside the plastic curtain to the center of the trough to take a sample.

The position of the organism in the trough indicates preference for a point in the gradient. By separating the upper and lower areas within the same concentration zone, other behavioral changes such as surface swimming of benthic organisms can be observed. The position of test animals in the upper or lower zones in the four areas is determined by the interruption of two pairs of infrared light beams and phototransistors per zone. A slightly modified version of previously described circuitry (Cripe et al. 1975) is used for the sensing system. AGARS uses RCA SG1009A infrared light-emitting diodes (LEDs) as light sources. The detector circuit (Fig. 2A) combines the signals from two sensors monitoring the same one-eighth area of the trough. An SN7440 integrated circuit (IC) could be used in place of the SN7400 to integrate the signal from all four sensors in the same concentration zone (the SN7400 monitors one pair of sensors in upper or lower half of area).

AGARS data are processed as follows. The status of the eight pairs of sensors is scanned by an inexpensive ($99) SC/MP microprocessor (National Semiconductor, ISP-SK/200) at 0.33-s intervals to determine the time and position of animals in the light beams. An animal resting in front of the beam continues to add to the cumulative time in the area. Cumulative results are sent hourly to a teletypewriter (Teletype Corp. Model 3320/3JC) that records totals in hexadecimal on a punched paper tape. Tape data are entered in a calculator/tape reader/X–Y plotter system (Hewlett Packard, Models 9825A, 2748B, and 9862A, respectively) and converted to decimal. Time spent in each zone is plotted, and results are analyzed statistically by a *t*-test corrected for uneven variance.

The basic SC/MP microprocessor is modified slightly for monitoring the sensors (Fig. 2). The jumper between J3 and J4 on the printed circuit board is broken. J3 is connected to an output of an SN74L86 gate (Fig. 2C); the eight bits of data from the trough are interfaced with an octal buffer, DM81LS95 (Fig. 2B). The ICs and resistors (Fig. 2B and C) fit in vacant predrilled holes on the printed circuit board. A program that scans eight pairs of sensors every 0.33 s and types hourly totals can be provided on request.

All 16 LEDs are wired in series to a 24 VDC power supply with a 300-ohm, 25-W, variable resistor in series to limit the current to approximately 40 mA. The sensor system requires a regulated +5 VDC power supply; the microprocessor needs both +5 VDC and −12 VDC. Three rechargeable lead–acid storage batteries (12V, 84 A·h) are used to supply power to the voltage regulators for the microprocessor so that possible loss of microprocessor memory, in event of power failure, is eliminated.

INITIAL AVOIDANCE STUDIES

Pinfish, (*Lagodon rhomboides*), SL = 6.5 cm, were seined from Santa Rosa Sound, Florida, near the Gulf

Breeze Environmental Research Laboratory. The pinfish were held in flowing, filtered seawater (22.7°–30.2°C, salinity 22–24‰) for 12 d before testing in AGARS.

The toxicant tested for avoidance was sodium hypochlorite, estimated as chlorine-produced oxidants (CPO) by amperometric titration with phenylarsine oxide buffered at a pH of 4.0. Stock solutions (distilled water and three sodium hypochlorite solutions at nominal concentrations of 310, 2100, and 3700 mg/L CPO) were metered into the four mixing boxes, Areas 1–4, respectively, at 1.0 mL/min by a Harvard Model 1203 Peristaltic Pump and silicone tubing. To determine concentration variability within each of the four areas, a gradient was established and water samples were analyzed from different points in each area over a period of several hours. Six points in Area 1, and 11 points in Areas 2–4, were sampled. CPO concentrations (milligrams per litre, mean ± SD) expected and found were as follows:

	Area			
	1	2	3	4
CPO expected	0.00	0.10	0.53	1.03
CPO found	0.00	0.02	0.09	0.28
	±0.00	±0.01	±0.03	±0.04

Four pinfish were monitored in the trough and time spent in each area was recorded for 9 d. Triplicate 200-mL samples were siphoned twice from the center of the four areas during each 24-h avoidance gradient exposure period. No toxicant was present on Days 1–3, 5, 6, 8, and 9. On Day 4 the gradient increased downstream and on Day 7 the gradient decreased downstream by addition of CPO in Area 1 only and by diluting with clean water in Areas 2–4. CPO concentrations (mg/L, mean ±SD) expected and found on Days 4 and 7 were as follows:

		Area			
Day		1	2	3	4
4	CPO expected	0.00	0.10	0.53	1.03
	CPO found	0.00	0.02	0.07	0.21
		±0.00	±0.01	±0.02	±0.03
7	CPO expected	0.52	0.26	0.17	0.13
	CPO found	0.09	0.04	0.02	0.01
		±0.01	±0.01	±0.00	±0.00

Trough temperatures during the 9-d test were between 28.5° and 29.0°C; salinity ranged from 22 to 26‰.

Avoidance response was determined by a *t*-test corrected for uneven variance on data from each area for a 24-h period, as compared with the distribution on the preceding day. An α level of 0.05 was used to determine significant decreases in time spent in an exposure area.

Results and Discussion

Both tables above indicate a substantial difference between expected and observed CPO values. This initial loss of CPO during chlorination of water is due to chlorine demand, and results from reaction with easily oxidized organic compounds, ferrous ions, sulfides, nitrites, and other reducing agents (Johnson 1978).

No CPO was added during the first 3 d of the test when fish were acclimated to the device (Fig. 3). On Day 1 fish spent most of the time in Area 1 where the current was the lowest. On Days 2 and 3 fish were distributed more uniformly throughout the four areas.

After CPO was added to the water on Day 4, pinfish spent significantly ($\alpha \leq 0.05$) less time than during the preceding day in Areas 3 (0.07 mg/L) and 4 (0.21 mg/L) where CPO concentrations were highest. No avoidance of Area 2 (0.02 mg/L) was evident. More residence time was recorded in Area 1 (control area) on Day 4, probably due to the avoidance of Areas 3 and 4. The avoidance threshold was thus indicated to be between 0.07 and 0.02 mg/L. Return to CPO-free water on Days 5 and 6 resulted in a continued high presence in Areas 1 and 2.

On Day 7, the second toxicant exposure day, Areas 1 (0.09 mg/L) and 2 (0.04 mg/L) were significantly ($\alpha \leq 0.05$) avoided; no avoidance was shown in Area 3 (0.02 mg/L), and an increased presence was recorded in Area 4 (0.01 mg/L), indicating a fish avoidance threshold of approximately 0.02 to 0.04 mg/L CPO. For Days 8 and 9 (CPO-free) fish spent more time in Area 4 than in any other area.

For 2 d following each toxicant exposure, fish continued to spend more time in the end that they had previously selected, perhaps indicating a conditioned response. This behavior was apparently overcome by reversing the gradient and does not appear to affect the usefulness of the apparatus. Total time spent in the light beams per hour is not fixed, since at any instant 0–4 light beams may be interrupted depending on the position of each of the four fish. Total time decreased on both exposure days and then increased on successive toxicant-free days. The reason for this was not determined but may be the result of toxicant-induced behavioral modifications such as decreased schooling size or decreased swimming activity.

Few data are available on avoidance thresholds for fish under saltwater conditions. Middaugh et al. (1977b) found that juvenile spot (*Leiostomus xanthurus*) tested at 19–22‰ avoided a total residual chlorination (TRC) of 0.05 mg/L at 15° and 20°C. No avoidance was observed at 0.03 mg/L (D. P. Middaugh, U.S. EPA Laboratory, Bears Bluff Field Station, P.O. 368, Johns Island, S.C. 29455, personal communication) at either 15° or 20°C; the avoidance threshold, 0.05–0.03 mg/L, was near the incipient LC50 level of 0.06 mg/L TRC. This threshold appears consistent with the range of 0.02–0.04 mg/L for pinfish in my AGARS test.

These data demonstrate the applicability of AGARS in examining an organism's long-term continuous avoidance of a pollutant. The versatility of the system, combined with the elimination of visual monitoring, presents advantages over other methods mentioned. The dimensions of the system place certain locomotor restrictions on the test organisms, as well as on their

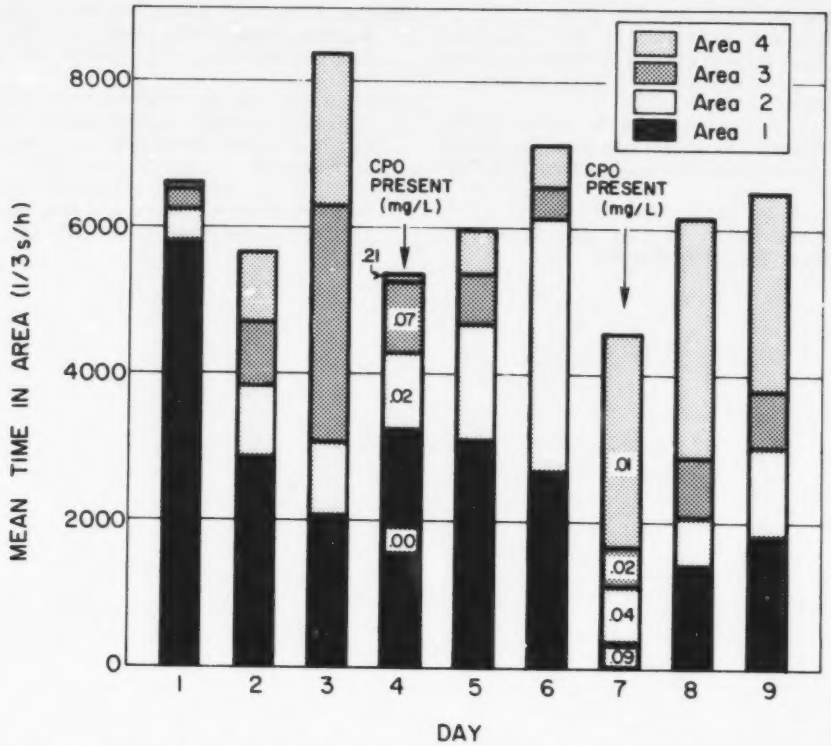

FIG. 3. Results of 9-d AGARS test with a group of four pinfish. Mean of 24-h totals of time spent in each of four areas of trough vs. elapsed time of test. On Days 1–3, 5, 6, 8, and 9 no toxicant was present. Chlorine produced oxidants (CPO) were present on Days 4 and 7. Mean CPO values are shown for two sampling periods per 24 h, with three replicates per sampling period.

maximum size. The system is a prototype, however, and can be enlarged by using more powerful lights and greater microprocessor memory capacity. In addition to several species of fish, baseline data have also been obtained with blue crabs, *Callinectes sapidus*, and penaeid shrimp. This system could also be used to test thermal or salinity preferences, provided the gradient is not so steep as to allow stratification of differing densities.

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